Sensory Quality and Histamine Formation during Controlled Decomposition of Tuna (Thunnus thynnus)

EMILIO I. LÓPEZ-SABATER,* JOSÉ J. RODRÍGUEZ-JEREZ, MANUELA HERNÁNDEZ-HERRERO, ARTUR X. ROIG-SAGUÉS, and MARIA T. MORA-VENTURA

Food Hygiene Department, Faculty of Veterinary Medicine, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain

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ABSTRACT

Histamine production was studied during controlled tunafish decomposition at 0, 8, and 20°C. The influence of the location of the anatomic section on the amount of histamine formed and the incidence of histidine decarboxylating bacteria were also considered. By the time of sensory rejection, histamine levels in tunafish sections stored at 0 and 20°C were still below the hazard levels and the allowable levels established by both the U.S. Food and Drug Administration (FDA) and the European Union. Toxic amounts were only formed after the tunafish was considered organoleptically unsuitable for human consumption. However, at 8°C, levels of histamine between 100 and 200 mg/100 g of fish were found before tuna reached the rejection point. Hence, physical appearance was not a good criterion for estimating the shelf life and especially the histamine-related health hazard when tuna was stored at 8°C, a common temperature in many home refrigerators.

Key words: Tunafish, histamine, flavor quality, storage stability, microbiological quality

Despite the high hygienic standards and food regulations established in European countries (1), the number of scombroid fish poisoning outbreaks reported has continued to increase. Although the exact mechanism of scombroid toxicity still remains uncertain, a high histamine content in fish flesh has long been considered the most probable cause of this type of food poisoning. Scombroid food poisoning ranks as the second most frequent cause of foodborne disease associated with finfish consumption in the United States (5) and is the most commonly recorded illness associated with eating fish in the United Kingdom (25). A lack of a relationship between histamine content and sensory attributes has been suggested to explain the high incidence of scombroid toxicity (20, 24, 26). Due to the relative thickness of scombroid fish skin, especially in the biggest tunas, sensory analysis for evidence of decomposition does not seem to be adequate.

On the other hand, Arnold and Brown (3), Frank et al. (9), Lerke et al. (11), and Löfberg et al. (12) reported that histamine levels varied greatly depending on the location of the portion of the fish. Histamine in tunafish showed an uneven distribution, with the highest content in the nape (anterior section) and the lowest in the tail (posterior section) (8). However, differences in histamine content have not been observed between the sides of each tuna section (4). Both the uneven distribution of histamine in the fish flesh and differences in sensitivity between individuals have been suggested to explain why some people became ill after eating tuna with a high histamine content and others who had eaten the same meal did not. Hence, it has been concluded that histamine content would not be a good index of tuna quality.

This work was conducted to investigate the stability of tuna stored at different temperatures (0, 8, and 20°C). The effect of anatomic section on histamine formation and sensory quality was also considered. Another goal was to identify the histamine formers isolated during this investigation and to assess their histidine decarboxylase activity both in a culture broth supplemented with 1.0% L-histidine-HCl and in tunafish muscle collected in sterile conditions.

MATERIALS AND METHODS

Storage conditions

A fresh tunafish (Thunnus thynnus) weighing 50 kg was purchased in the wholesale fish market of Barcelona and immediately transported to the laboratory in crushed ice. Three anatomical sections (anterior, middle, and posterior) from the tuna were examined, each of which was further cut into three pieces. Individual pieces (approximately 10 to 13 cm thick, 3 to 4 kg weight and including skin) were placed in separate sterile polyethylene bags (Eurotube®). One piece from each anatomic area was stored at each of the selected temperatures, 0, 8, and 20°C. Samples were periodically taken for sensory, bacteriological, and chemical analyses, at 12-h intervals in sections stored at 20°C, daily in
sections stored at 8°C, and every 3 days in sections maintained at 0°C.

Sensory evaluation

Samples of tunafish muscle taken at regular intervals were placed in individual trays, and their sensory quality was evaluated by three trained panelists in a well-ventilated room with natural daylight. No reference (high quality tuna) was used by panelists for comparison to stored fish. Quality was assessed using Soudan’s scale (22). This hedonic table includes 11 attributes. However, not all of them need to be rated for each evaluation. Only those characteristics readily available in tuna sections (appearance of the skin and peritoneum, slime on the skin, general odor and texture, color of muscle) were scored, from 1 for the best quality to 6 for very poor quality, and the mean of the scores obtained for each aspect was used as the sensory score. Tuna was considered unsuitable for human consumption and, therefore, rejected when a final score of 3 or higher was obtained in the organoleptic assessment.

Enumeration of bacteria

Before the sensory evaluation, samples of tuna muscle were obtained for both microbiological and chemical analyses. The bacterial analyses involved counting colonies of total mesophilic aerobic flora, psychrotrophic flora, Enterobacteriaceae, and total coliforms by standard methods (2). Histamine-forming bacteria were enumerated by the MPN technique (27).

Histamine-forming isolates

Histamine-forming bacteria were isolated using a modified Niven’s medium (24). Purple colonies in Niven’s medium were picked and maintained on Trypticase soy agar (TSA) (Difco) slants containing 0.1% L-histidine-HCl (Aldrich Chemical Co.) at 2°C until use. Isolates were identified using the PASCO Gram Negative and Positive Identification System (Difco) and descriptions in Bergey’s Manual of Determinative Bacteriology (8th ed.).

Histidine decarboxylase activity of all isolates was assessed in a formulated broth containing 1.0% L-histidine-HCl after 18 h of incubation at 37°C (13). Histidine decarboxylase activity in tuna meat

Fifteen bags, each containing 450 g of aseptically collected tunafish muscle, were prepared. In a vertical laminar flow hood, the tuna skin was washed with ethanol-acetone (1:1) and peeled away from the fish, avoiding any contact between skin and muscle. Small pieces of muscle tissue were cut and placed in sterile polyethylene bags (Eurotube®).

Four species (Morganella morganii, Klebsiella oxytoca, Serratia marcescens, and Plesiomonas shigelloides) isolated in the preceding study were selected to test their ability to form histamine in tunafish meat at three different temperatures (0, 8, and 20°C). Preceding studies have shown that histidine-decarboxylating bacteria only accounted for a small proportion of tunafish contamination at the three selected temperatures. By the end of storage at 20°C, histidine decarboxylase activity of all isolates was assessed in a formulated broth containing 1.0% L-histidine-HCl after 18 h of incubation at 37°C (13). Histamine in a 10-g portion of tunafish flesh was extracted with 50 ml of 0.4 N HClO₄ and measured by using an enzymatic method (14). Briefly, the method is based on the sequential action of the diamine oxidase and peroxidase enzymes upon the histamine present in the fish extract, with final formation of crystal violet which is measured by colorimetry at 596 nm.

Statistical analysis

Data were analyzed by using the statistical package SPSS/PC+ (SPSS Inc., Chicago, IL). Analysis of variance was carried out to study differences in relation to the anatomic area and multiple regression analysis was used to evaluate the influence of microbial groups on histamine formation during tuna spoilage.

RESULTS AND DISCUSSION

Bacteriological results during storage

All microbial groups increased in number during storage of tunafish at the three temperatures. None of microbial groups considered showed significant differences (P ≥ 0.05) in relation to the anatomic area. As expected, decomposition of tunafish at temperatures between 0 and 20°C was associated mainly with psychrotrophic bacteria. Enterobacteriaceae and total coliforms did not seem to be part of the normal microbiota of fresh fish. They were not detected until 3 days of storage at 0°C and 24 h at 8°C (data not shown).

Counts of histamine-forming bacteria as functions of the anatomic area and temperature are summarized in Figure 1. Counts were similar in the three tunafish sections for a given temperature. At 0°C histamine-forming bacteria were not observed until 9 to 12 days of storage. In samples stored at 8°C histidine-decarboxylating bacteria were detected after 2 to 3 days depending on the section; such bacteria were detected between 12 and 24 h at 20°C. Although their occurrence was higher in samples stored at 20°C, histidine-decarboxylating bacteria only accounted for a small proportion of tunafish contamination at the three selected temperatures. By the end of storage at 20°C, histidine decarboxylase activity of all isolates was assessed in a formulated broth containing 1.0% L-histidine-HCl after 18 h of incubation at 37°C (13). Histamine in a 10-g portion of tunafish flesh was extracted with 50 ml of 0.4 N HClO₄ and measured by using an enzymatic method (14). Briefly, the method is based on the sequential action of the diamine oxidase and peroxidase enzymes upon the histamine present in the fish extract, with final formation of crystal violet which is measured by colorimetry at 596 nm.

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differences in the histamine content depending on the anatomic fish section. Lerke et al. (11) reported that a tunafish sample could contain a histamine content as much as four times higher than another taken 3 cm away. Our results seem to differ from these earlier works and show an even formation of histamine under the experimental conditions described. From this standpoint, different sensitivity to histamine between individuals, instead of the uneven distribution of histamine in the fish flesh, could better explain why some people become ill and others do not after eating the same meal containing tuna with a high amount of histamine.

Histamine was not detected until day 12 in sections stored at 0°C (Figure 2). Then the concentration of histamine began to increase, reaching levels higher than the limits

were probably influenced by the selection of colonies studied. In our experience, the histidine decarboxylase broth formulated by Yamani and Unterman is more selective and reliable than Niven’s agar medium. Only microorganisms with a high histidine decarboxylase activity, such as Morganella morganii, Klebsiella oxytoca, or Klebsiella pneumoniae, caused the pH indicator to change color, so false-positive results were very unlikely.

Histamine content

Histamine distribution was uniform ($P \geq 0.05$) throughout all fish sections during our study. Similarly, Middlebrooks et al. (15) did not report significant differences in the amount of histamine formed in tunafish sections stored at 30°C for 48 h. However, these results are in contrast to those of some previous studies (9, 12), which indicated great

FIGURE 1. Histamine-forming bacteria count during tunafish decomposition at 0°C (A), 8°C (B) and 20°C (C) depending on the anatomic section studied: anterior (●), middle (▲), and posterior (■).

FIGURE 2. Sensorial quality and histamine content in tunafish sections (anterior (A), middle (B), and posterior (C)) stored at 0°C. Sensorial quality was assessed using a 1 (fresh) to 6 (spoiled) Soudan’s scale.
established by the FDA (50 mg/100 g) on day 18 in the middle (115 mg/100 g) and posterior section (199 mg/100 g) and on day 21 in the anterior section (98 mg/100 g). Similar results have been reported in mackerel (144 mg/100 g) after 12 days of storage at 2°C (17). After reaching a maximum concentration on day 18, histamine content began to decrease in middle and posterior sections. The presence of microorganisms with histaminase activity has been suggested to explain this observation (23). At the time of rejection (sensory score >3) the histamine content in tuna stored at 0°C was negligible (Figure 2). Toxic amounts of histamine were formed only after deterioration of tuna made it unsuitable for human consumption. These results suggest that, when tuna is properly stored at low nonfreezing temperatures, histamine formation would not have to represent a serious health risk to consumers, unless the tuna was mishandled previously.

Decomposition and histamine formation in samples stored at 8°C were faster than at low nonfreezing temperatures (Figure 3). Before 24 h histamine formation remained negligible in the three anatomic sections studied, but just after that it began to increase rapidly, reaching concentrations higher than the FDA limit on day 4 in the anterior (141 mg/100 g) and middle sections (150 mg/100 g) and on day 3 in the posterior section (169 mg/100 g). The concentrations of histamine observed here are similar to values reported by Cattaneo and Cantoni (7) in tuna stored at 6°C for 4 days (88 to 278 mg/100 g) and by Park et al. (18) in mackerel and sardine stored at 10°C for 4 to 5 days (130 and 250 mg/100 g, respectively). The organoleptic rejection point was reached on day 6 in all sections. At this time the tuna was considered unpleasant and inedible. Figure 3 shows the relationship between histamine formation and sensorial score in sections stored at 8°C. Before decomposition was evident by sensory criteria, enough histamine had accumulated to cause a health problem according to the toxic limits (100 mg of histamine in 100 g of fish) reported by Arnold and Brown (3). When the sensory evaluation gave a score of 3 (limit of rejection) or higher, histamine content had increased to 281, 131, and 172 mg/100 g in the anterior, middle and posterior tuna sections, respectively. Therefore, storage of tuna at 8°C and consumption of such food after extended refrigeration appears to be particularly hazardous to consumers. These findings are in agreement with the results of Ramesh et al. (21), who reported that dark-meat fish species stored at refrigerated temperatures only developed external signs of decomposition when histamine content in the muscle was higher than 650 to 900 mg/100 g.

Both deterioration and histamine formation occurred very quickly in the three sections maintained at 20°C (Figure 4). Histamine formation was negligible during the first 12 h of storage, but after the initial appearance of this biogenic amine the rate of increase was extremely fast. Although histamine began to increase slightly faster in the anterior section, after 48 h histamine content was fairly similar and significant differences were not observed among the anatomic sections considered. The fastest formation of histamine in the anterior section could be related to a major incidence of enterobacteria contamination from the intestinal tract. At this temperature decomposition of tuna progresses faster than histamine formation. Thus, tuna was considered overtly spoiled (sensory score >3) and therefore unsuitable for human consumption before histamine content reached toxic levels.

Histamine-forming bacteria
A great number (374 isolates) of histamine-forming strains were isolated during the study. Their identity and histidine decarboxylase activity are reported in Table 1. Most of the isolates were gram-negative bacteria. Only eight gram-positive strains, all identified as Bacillus spp., were isolated from Niven agar medium. However, although they had changed the pH indicator from green to purple in the Niven’s medium, all of them failed to form histamine when
their enzymatic activity was measured in a culture broth supplemented with free histidine. Similarly, Pogarzelski (19) isolated Bacillus species from wines containing histamine, although none showed histidine decarboxylase activity. Some gram-negative bacterial species initially isolated from tuna using the Niven agar medium, such as Acinetobacter anitratus, Pseudomonas fluorescens/putida and Cedecea lapagei, also failed to form histamine in the experimental conditions described. However, P. fluorescens/putida has frequently been isolated and identified as a weak histamine-former in fish (115).

Morganella morganii, Klebsiella oxytoca, Citrobacter freundii, and Enterobacter agglomerans were mainly isolated from sections stored at 20°C. In contrast, Hafnia alvei, Serratia liquefaciens, Cedecea lapagei, and Enterobacter intermedium were more prevalent at 8°C. Klausen and Huss (10) have ascertained that although M. morganii shows a rapid growth rate when incubated at 20 to 25°C, it does not grow at lower temperatures. Otherwise, most of the M. morganii isolates showed a powerful histidine decarboxylase activity (>300 mg/100 g after 18 h incubation at 37°C). In some instances, other isolates such as C. freundii, E. agglomerans, E. cloacae, H. alvei, and S. marcescens were able to form more than 100 mg/100 g. The remaining isolates were all identified as weak histamine formers. Only those microorganisms able to produce more than 100 mg/100 g of histamine during a short incubation would be of concern as a potential toxicological hazard.

Histidine decarboxylase activity in tuna meat
Neither histamine formation nor bacterial growth was observed in any of the control samples at the three temperatures.
FIGURE 5. Growth (■) and histamine formation (●) by Morganella morganii (A), Klebsiella oxytoca (B), Serratia marcescens (C) and Plesiomonas shigelloides (D) in tunafish meat at 20°C.

FIGURE 6. Growth (■) and histamine formation (●) by Morganella morganii (A), Klebsiella oxytoca (B), Serratia marcescens (C) and Plesiomonas shigelloides (D) in tunafish meat at 8°C.
tures considered (0, 8, and 20°C). This result confirms the bacterial origin of histamine formed in fish. In the absence of bacterial contamination, no histamine can be formed. Although the four strains used in this study grew well in tuna samples stored at 20°C, only M. morganii (15 mg/100 g) and K. oxytoca (14.3 mg/100 g) produced detectable levels of histamine after 18 h (Figure 5). However, the amount of histamine formed by both species was considerably lower than that observed in the culture broth. The difference could be due to the different temperature and especially the lower histidine availability in the tunafish muscle. Although tuna contains a high amount of histidine in its muscle tissue, in some instances higher than 1%, tissue histidine probably would not be so easily available for bacterial metabolism as in a culture broth. Thus, these results suggest that, in spite of a high inoculum (10⁶ cells per g) of a powerful histamine former such as M. morganii or K. oxytoca, which could be present in raw tuna meat stored at room temperature, other factors are required to form high amounts of this biogenic amine in a short time. Probably the presence of some psychrotrophic bacteria, such as pseudomonads or alteromonads, frequently related to fish spoilage and known as active proteolytic microorganisms, would be required to release histidine from tuna proteins and then to increase the formation of histamine. Furthermore, Behling and Taylor (6) have pointed out that the maximum histidine decarboxylase activity was observed during the late logarithmic phase of growth. In our study, none of the four strains reached this point after 18 h of storage and this could also affect their ability to synthesize the enzyme histidine decarboxylase.

The four strains showed similar growth and histamine formation in tuna samples stored at 8°C (Figure 6). After 84 h of storage, 2.3, 2.8, 3.1, and 4.3 mg of histamine per 100 g were observed in tuna samples inoculated with S. marcescens, K. oxytoca, P. shigelloides, and M. morganii, respectively. In contrast, however, none of the four strains was able to grow and to form detectable levels of histamine after 7 days of storage at 0°C. Thus, although other factors and microorganisms can be involved in histamine formation, it can be stated that even though a high count (10⁶ cells per g) of M. morganii or K. oxytoca (probably the two most powerful histamine-formers known) were present in tuna, if a proper refrigeration process were applied (0°C), no histamine would be formed by them. Hence, storage of fish at 0°C or lower temperatures would be the best way to control histamine formation in fish.

Conclusion

In conclusion, although decomposition occurs more quickly than histamine formation in fish stored at 0°C and at room temperature (20°C), and probably at higher temperatures, unfortunately when tunafish is stored at refrigerated conditions (8°C) histamine formation continues and the physical appearance is not a good criterion to evaluate the histamine-related health hazard, which could explain the high incidence of this type of food poisoning.

Thus, to prevent histamine-related illnesses and considering that refrigeration at 8°C (a common temperature in many domestic refrigerators) does not prevent histamine formation, once tuna has been purchased and even though it is stored at refrigerator temperatures, it would have to be consumed as soon as possible and surely within 3 days. Longer storage may result in a rapid rise in histamine content and in a potential threat to consumers’ health.

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